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Optimization of Triploidy Induction and Growth Performance of Clarias Anguillarias (African Catfish) using Cold Shock

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Abstract

Chromosome manipulation produce polyploidy, which improves fish yield in aquaculture. Triploid hatchlings were obtained from the egg and sperm of Clarias anguillarias fertilized artificially and exposed to cold shock at 5^0C for 20 minutes, three minutes after fertilization. A total number of 20 juveniles from treatment A:(Diploid) and treatment B: (Triploid) were collected two weeks after hatching. Blood samples for erythrocyte measurements were obtained after cutting the caudal fin of the samples. T-test results was significant ($P<0.05$) for erythrocyte nucleus volume (where the value for treatment 'A' was 0.64±0.03 and 1.15± 0.18 for treatment 'B') and erythrocyte cell volume, (where treatment 'A' was valued at 13.20±0.13 and treatment 'B' at 15.27±0.66). One way analysis of variance revealed that treatment 'B' produced higher mean weight gain (166.66±53.44g), than treatment 'A' whose value was 50.40±3.03%. However, a higher hatching rate was realized from treatment 'A' having value of 77.53±2.19%, than 50.40±3.03% for treatment 'B'. This study has shown that bigger fish was produced when triploid than diploid catfish were reared. Thus the research has provided a better alternative to bigger and more fish production in aquaculture. It also showed that erythrocyte measurement was reliable for the determination of polyploidy levels.

Keywords: *Triploidy, Clarias anguillarias, Erythrocyte measurements, Cold shock, Polyploidy*

1. Introduction

Polyploidy is an occurrence in which more than two numbers of chromosomes are present in an organism. Chromosomes numbers of 3n are referred to triploids, 4n: are tetraploids, while 5n are pentaploids. Chromosome numbers have been altered through cold, heat, pressure or chemical shocks. Such alterations have been associated with advantageous features such as increased size, hardiness, and resistance to diseases (Lawson and Ishola, 2010). Beyond, the basic goal governing this technology are: to produce sterile fish which prevents early maturation, encourage the production of larger fish, improvement of carcass quality and the utilization of exotic species both in fish farming and fisheries management (Lawson and Ishola, 2010). Triploids have been created in fishes using thermal shocks (Dunham *et al.,* 2003). Eyo *et al.* (2003), induced triploidy in rainbow trout by raising the temperature in which the newly hatched eggs were incubated at 20^0C for 10 minutes beginning from 20-40 minutes after fertilization. Eyo *et al.,* (2003) observed that triploid channel catfish had a better dress out percentage than diploid fish as a result of lack of gonadal development. Hammed *et al.,* (2010), reported that shock duration of 25 minutes at 0^0C gave the best results for *Clarias gariepinus.* Giuliano and Evoy (2006) stated that exposure for five

minutes post fertilization was enough to induce 100% triploidy in *Rhamdia quelen*. Lawson and Ishola (2010) reported that cold shock treatment showed better growth rate and lower survival rate compared to diploid *Clarias gariepinus*. Normala *et al.,* (2010) reported that triploid catfish had lower hatchability rate and survival rate, but higher growth rate compared to diploid catfish. Gheyas *et al.,* (2001), reported that induce triploidy in newly fertilized eggs of *H. fossilis* using cold shock duration for 10 minutes at 20^0C applied 3 minutes after fertilization was the best. Gima (2009) reported that triploid grew better than diploid flathead catfish although the diploids were more aggressive than the triploids. Venkatachalam *et al.,* (2012) reported that the extent of triploidy was measured through Erythrocytes Nucleus Volume (ENV). In diploid fish, the ENV was 11.7±1.7μm^3 where as in triploid fish it was 17.2±3.2 μm^3. Koedprang and Na-Nakron (2000), recorded high hatchability of 72.5% in the production of triploid silver barb when cold shock was applied for a duration of 10 minutes while, Hammad *et al.,* (2010) recorded 55% hatchability in triploid induced eggs using cold shock method for a duration of 25 minutes at 0^0C. The present study is aimed at investigating the growth rate of triploid hatchlings as well as ascertain cytogenetically, their ploidy levels through erythrocyte measurement.

2. Materials and Methods

2.1 Procurement and Selection of Broodstock:

A total number of three (3) healthy broodstocks of *Clarias anguillaris* (one male and two females) used in this study were procured from Hotefe farm, Asaba, Delta State, Nigeria. They were eighteen (18) months old, weighing between 3 to 4 kilogram's. Care was exercised when selecting healthy male and female brooders.

2.2 Administration of Ovaprim:

The female brooder (*Clarias anguillaris*) was injected with Human Gonadotropin Hormone (Trade mark: ovaprim) at a dosage of 0.5 ml per kilogram fish body weight. The injection was done intramuscularly above the lateral line just below the dorsal fin. Injected brood were kept in separate bowls of 1m^3 and covered with netting piece to prevent them from jumping out. The temperature of the water holding the fish was measured with mercury in glass thermometer and the corresponding latency period calculated was between eight to ten hours at 28.3^0C according to (Herbst, 2002).

2.3 Procurement of Milt:

The milt used was procured by sacrificing the male. Prior to this action, physiological solution was prepared by dissolving nine grams of salt (NaCl) in one litre of water according to Lawson and Ishola, 2003.

2.4 Stripping of Eggs

The first step taken during the stripping process was to mop the body of the female brooder with a towel, to prevent the eggs from coming in contact with water which may consequently seal up the micropyle opening and prevent fertilization. Gentle pressure was applied on the abdomen of the female brooder and the ovulated eggs that ooze out freely from the genital opening was collected in a stainless steel bowl where the eggs were subjected to cold shock. Control experiment was also provided according to (Lawson and Ishola, 2003).

2.5 Fertilization of Eggs:

The incubation bowls and cold shock medium were prepared prior to fertilization. Each of the bowls was filled with 10 litres of clean water. The water was aerated with electric air pumps to which hose and air stones were connected. Mosquito net (kakabans) was also laid in the water so that as the fertilized eggs cross through into the bowl, where they were distributed evenly, the unfertilized eggs were retained/suspended by the net and later discarded. Milt was poured on the eggs and mixed by shaking the containers gently according to (Lawson and Ishola, 2003).

2.6 Post Fertilization Treatments:

The fertilized eggs were separated into two treatments (A and B). Treatment A was Triploidy, and treatment B was pure strains of *C. anguillaris*. Three (3) minutes old fertilized eggs were subjected to cold shock for treatment A and A1 at 5^0C for 20 minutes Cold shock of fertilized eggs was applied in thermostatically controlled water bath (model FDPGH, Techne Cambridge, Ltd UK). Fertilized eggs were removed from the cold medium and placed in the plastic bowls for normal incubation at 27.4^0C (Lawson and Ishola, 2003). Water quality was checked and maintained throughout for pH, dissolved oxygen and temperature.

2.7 Hatching of Fertilized Eggs:

Commencement of hatching was noticed after 22.00 hours and 22.5 hours of incubation in control and cold shock experiments respectively (Lawson and Ishola, 2003).

2.8 Larval Rearing:

Larval rearing was carried out by placing the hatchlings, each of cold shock and control into nursery tanks. Within the first three days, the larvae were nourished from their yolk sac. After four days, the fry were fed with artemia sp for two weeks according to (Lawson and Ishola, 2003).

2.9 Fry Rearing:

Two weeks after hatching, twenty fries from cold shock and control experiments were stocked into the culture receptacles (concrete tanks) in duplicates. They were fed on Coppens twice a day at 5% body weight according to (Lawson and Ishola, 2003).

2.10 Nutrient Utilization Parameters

- The Mean Weight Gain (%), was calculated according to Pangni *et al.*, (2008) thus; MWG (%) = $\frac{\text{Final mean weight}}{\text{Initial mean weight}} \times 100$
- The Mean Length Gain (%), was calculated according to Lawson and Ishola, (2003) thus; MLG (%) = $\frac{\text{Final mean length}}{\text{Initial mean length}} \times 100$
- The Specific Growth Rate (SGR) was calculated according to Lawson and Ishola, (2003): SGR(%/day) = $100 \times \frac{[\ln(\text{Final body weight}) - \ln(\text{Initial body weight})]}{\text{Rearing period in days}}$

Where "ln" represents natural logarithm

- Survival rate (SR) was calculated according to Pangni *et al.*, (2008)
  SR (%) = $\frac{\text{Total fish number harvest}}{\text{Total fish number stocked}} \times 100$

- Hatchability rate (HR) was calculated according to Lawson and Ishola, (2003): HR (%) = Total eggs number fertilized X 100
  Total eggs number hatched

2.11 Water Quality Monitoring

Water quality parameters such as dissolved oxygen, pH and water temperature required for growth and other biological processes were monitored weekly. Water in the culture receptacles were changed daily and aerated with air pumps throughout the period of study, to ensure high water quality and to prevent stress (Lawson and Ishola, 2003).

2.12 Polyploidy Optimization through Erythrocyte Measurement

A comparative measurement of erythrocytes and nuclei from diploid and triploid treatments were carried out to observe the effect of triploidization on cell size. Blood samples were collected from seven triploid and seven control groups post juveniles. Smears were produced on glass slides and fixed for two minutes in absolute methanol (Gheyas *et al*., 2001). The smears were subsequently stained with Wright's blood stain for 10 to 15 minutes, washed in distilled water, air dried and finally mounted in DPX (a mixture of Distrene: Dibutyl Phthalate: Xylem). Twenty-five erythrocyte cells from each treatment (major axes A and major axes B) with their nuclei were measured with an eye piece micrometer under 100 times magnification according to (Gheyas *et al*., 2001). Volumes of the cells and their nuclei were computed using the formulae given by Felip *et al*., (2001a), as follows:

$V_{erythrocyte} = 4/3 \times \pi \times (A/2) \times (B/2)^2$

$V_{nucleus} = 4/3 \times \pi \times (a/2) \times (b/2)^2$

Where: A = major axis of erythrocyte, B = minor axis of erythrocyte, a = major axis of nucleus and b = minor axis of nucleus.

2.13 Statistical Analysis

Data obtained from the production experiment were subjected to one-way Analysis of Variance (ANOVA) using the SPSS software and difference between means were separated using Duncan's Multiple Range Tests. Data obtained from erythrocyte measurement were analysed using student T-test at 95% level of significant.

3. Results

The mean production parameters for both treatments as presented in Table 1, revealed that triploid treatments were significantly higher than diploid treatments for all parameters, except for survival rate where equal values were obtained for both treatments. Triploid treatment recorded higher mean weight gain (166.66±53.44g) than diploid treatment, whose value was (105.93±32.23g). The same table showed that triploid treatment recorded higher specific growth rate value of (5.70±2.27%), than diploid (3.14±0.64) treatment. However, survival rate data was not significantly different ($P > 0.05$) for both treatments, although. the hatchability value for both treatments were significantly different ($P < 0.05$). Incidentally, a lower hatching rate was obtained for triploid treatments with a value of 50.40±3.03% whereas a higher hatching rate was obtained for diploid treatment having a value of 77.53±2.19% (Table 1).

Table 1. Show values for mean production parameters of *Clarias anguillaris*

Treatments	WG (g)	LG (cm)	SGR (%)	SR (%)	HR (%)
A (Triploid)	166.66±53.44[a]	19.43±2.81[a]	5.70±2.27[a]	100.00±0.00[a]	50.40±3.03[b]
B (Diploid)	105.93±32.23[b]	16.27±2.37[b]	3.14±0.64[b]	100.00±0.00[a]	77.53±2.19[a]

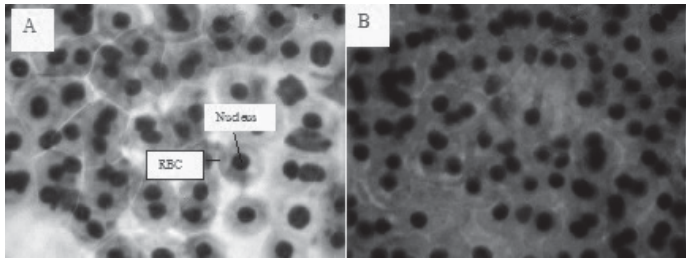
Values with the same superscript on the same column are not significantly different at ($P > 0.05$) confidence limits. Key: WG = weight gain, LG = length gain, SGR = specific growth rate, SR= survival rate, HR = hatchability rate and ± standard error of the mean.

Ploidy level by cold shock was assessed by erythrocyte measurement, where normal diploid recorded a cell volume of 13.20±0.13 ·m^3 whereas triploid cell volume was 15.27±0.66··m^3 (Table 2). Plates A and B represents erythrocytes of diploid and triploid individuals respectively. The plates clearly show that nuclei of triploid erythrocytes (plate A) are considerably larger than those for diploids (plate B).

Table 2: Erythrocyte Measurement of Diploid and Triploid *Clarias anguillaris* (cell volumes in cubic micrometer-·m^3)

Types Of Cells	2n (Diploid)	3n (Triploid)	Ratio 2n/3n	Significant level
ECV (·m^3)	13.20±0.13	15.27±0.66	1.16	P<0.95
ENV (·m^3)	0.64± 0.03	1.15±0.18	1.92	P<0.95

Where; ECV = erythrocyte cell volume, ENV = erythrocyte nuclear volume and ± = standard error of the mean



Plates A and B shows the nucleus of triploid and diploid blood specimen of *Clarias anguillaris* erythrocytes at x100 magnification.

Atmospheric temperature varied between 23.1 and 27.3^{0}C, while water temperature values ranged from 26.1 to 27.5^{0}C. Values of dissolved oxygen concentration revealed a variation from 2.2 to 6.01 mg/L, while that realized for the control treatments varied between 3.4 to 8.9 mg/L. Hydrogen-ion-concentration (pH) for both treatments ranged between 7.01 to 8.22.

4. Discussion

4.1 Shock Application and Effects

Cold shock treatment adversely impacted on fertilized egg, resulting in bent trunk observed in fries exposed to such treatment. Similar observation has been reported by Manickam (1991), Aluko et al*.,* (1997) and Lawson and Ishola (2010) in *C. gariepinus*. These authors suggested that the abnormality could be due to chromosome malfunction. It was generally thought that warm water species were more susceptible to cold than heat shock (Gheyas *et al*., 2001), whereas heat shock was more effective for cold water species. The present experiment supports the contention that

because *Clarias* species are inhabitant of warm climate they were more highly susceptible to cold shock treatments. The occurrence of mosaic off springs (having different ploidy levels in the cells of the same individual) is a frequent observation in studies regarding triploidy induction (Gheyas *et al*., 2001; Herbst, 2002; Eyo *et al*., 2003 and Lawson and Ishola, 2010). It is most probably the result of exposure of the eggs to shock temperature after fertilization. In our experiment all suboptimal shock showed diploid/triploid mosaicism in the embryos of treated groups.

4.2 Growth Parameters

Fish growth and their consequent increase in biomass are of major interest to the fish culturist, the fish nutritionist and fishery biologist. Lawson and Ishola (2010) explained that growth of farmed fish was best described in terms of weight rather than length since the ultimate product is usually sold in terms of weight. According to Karlmarx and Sanjeeviraj (2005) chromosome engineering has immediate application in fish farming, because it improves strains of catfish for better growth, assures disease resistance, allows for higher fecundity and increases their tolerance to environmental conditions. The present experiment verified this contention and showed that triploid treatments had higher mean weight gain, than diploid treatment. This observation was in line with those reported by Gheyas *et al*., (2001); Herbst (2002); Eyo *et al*., (2003) and Lawson and Ishola (2010). Also, the realization that values for specific growth rate were higher in triploids than diploids agrees with the reports of Lawson and Ishola (2010) who also recorded high specific growth rate for triploid *Clarias gariepinus.*

4.3 Hatching and Survival Rates

The hatching and survival percentages in cold shock treated groups were considerably lower than those of control group in the present experiment. Such lower hatchability of triploid individuals compared to diploid have been reported by other authors including Chrisman *et al.,* 1983, Krasznai *et al.,* 1984a*,* Solar *et al.,* 1984, Gheyas *et al*., 2001; Herbst, 2002; Eyo *et al*., 2003; Giuliano and Evoy 2006; Lawson and Ishola, 2010 and Normala *et al.,* 2010). Moreover, the hatching, survival and triploidy induction rates varied considerably among different lots of eggs subjected to the same cold shock. Similar results have also been reported in other species and have been attributed to factors such as egg quality differences or the susceptibility of eggs from different origins to shock treatments (Lou and Purdom 1984, Johnstone 1985, Shelton et al. 1986, Ezaz et al.1998). Malison et al., (1993), however, suggested the wide range of ambient water temperature from which brood fish were captured as the probable cause for such variation in perch (*Perca flavescens*). For *C. anguillaris*, such factors in addition to egg quality could have been important. Moreover, the variation in the water temperature during the incubation period under ambient conditions might have also contributed to difference in hatching and survival rates. However, the survival rate data was not significantly different ($P>0.05$) for each treatments. It was constant all through the experiment with 100.00±0.00% value for both treatments. This finding disagrees with those of Gheyas *et al*., (2001) who had 95 to 97% survival for triploid Stinging Catfish (*Heteropneustes fossilis*); Lawson and Ishola (2010) reported 63% survival for triploid *Clarias gariepinus* whereas the study of Herbst (2002) observed 26% both for tetraploid Zebra fish (*Danio rerio*) and Nile Tilapia (*Oreochromis niloticus*).

4.4 Erythrocyte Measurement

The effect of triploidy on the erythrocyte cellular and nuclear volume dimensions from blood smears were highly significant in the present study. Unlike mammals, erythrocytes in fish species have a nucleus. Erythrocytes and their nucleus are assumed to have an ellipsoisal shape (Felip *et al*., 2001a). Erythrocyte measurement has been used by many workers (Swarup 1959, Cherfas

1966, Wolters *et al*., 1982, Krasznai *et al*., 1984b, Lemoine and Smith 1980, Sezaki *et al*., 1985, Don and Avtalion 1988, Humayun *et al.,*1994, Felip *et al*., 2001a, Felip *et al*., 2001b, Gheyas *et al*., 2001, Cal *et al.,* 2005 and Normala *et al.,* 2010) to determine the ploidy level in fish. In respect to increase in chromosome size, the size of the erythrocytes particularly that of their nuclei increased. These workers suggested different variable measures of erythrocytes and their nuclei to be important in ploidy determination. According to Wolters *et al.,* (1982) mean major axis was the best single variable in channel catfish. Penman *et al.,* (1987) also found the same variable useful in distinguishing triploid and diploid fish. Majority of other workers however emphasized on nuclear volume. Triploidization generally increased nuclear volume by 1.5 times (Swarup 1959, Beck and Biggers 1983, Purdom 1972 and Gheyas *et al*., 2001). In the present investigation, both nuclei and erythrocytes of tripliods were larger. Nuclear volume, however, showed a 1.92 times increase in triploids instead of the usual 1.5 times in other species. Significantly greater value ($P < 0.95$) for all variables (major axis, minor axis and volumes) in triploid *C. anguillaris* indicated that any of the parameters could be used for identification of ploidy in this species. This finding was in line with those of Felip *et al*., (2001a), Felip *et al*., (2001b), Gheyas *et al*., (2001), Cal *et al.,* (2005) and Normala *et al.,* (2010).

5. Conclusion

This study has proved that triploidy could be produced in *C. anguillaris* by administering cold shock treatment, 3 minutes after fertilization at 5^0C for 20 minutes. This study has also shown that erythrocyte measurement can be used to ascertain ploidy level in *C. anguillaris.*

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